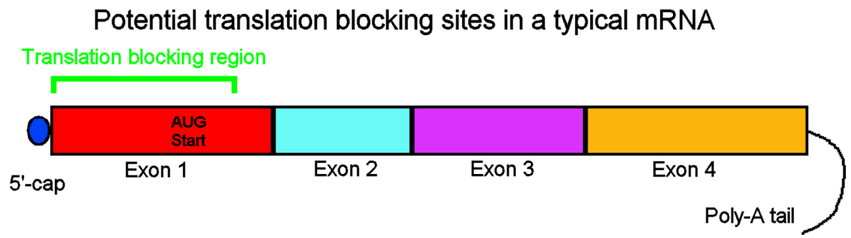
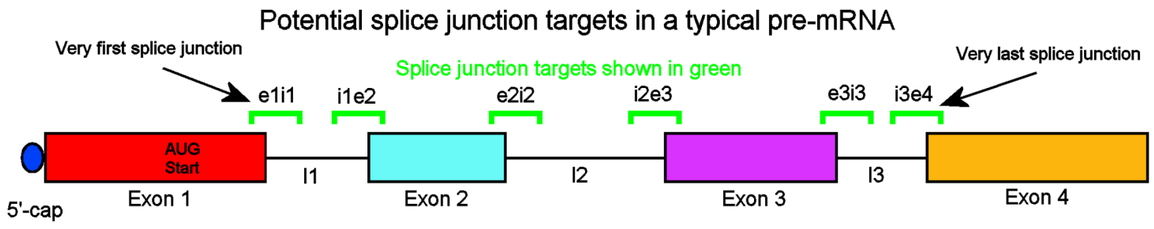
Targeting mRNA translation or pre-mRNA splicing

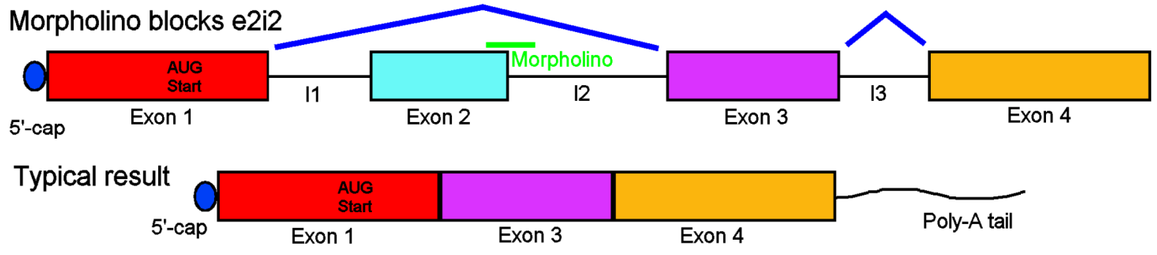
To block translation of a particular mRNA, a Morpholino oligo is made complementary to a target in the 5ôUTR through the start of coding sequence, as long as part of the Morpholino binds at or upstream of the start codon. When the small subunit of the ribosome, as part of the initiation complex, moves from the 5ôcap or an internal ribosome entry site toward the start codon, a Morpholino bound in its path can halt its progression and prevent the mature ribosome from forming. Note that if there is an intron in the 5ôUTR, the start codon will appear in a later exon.



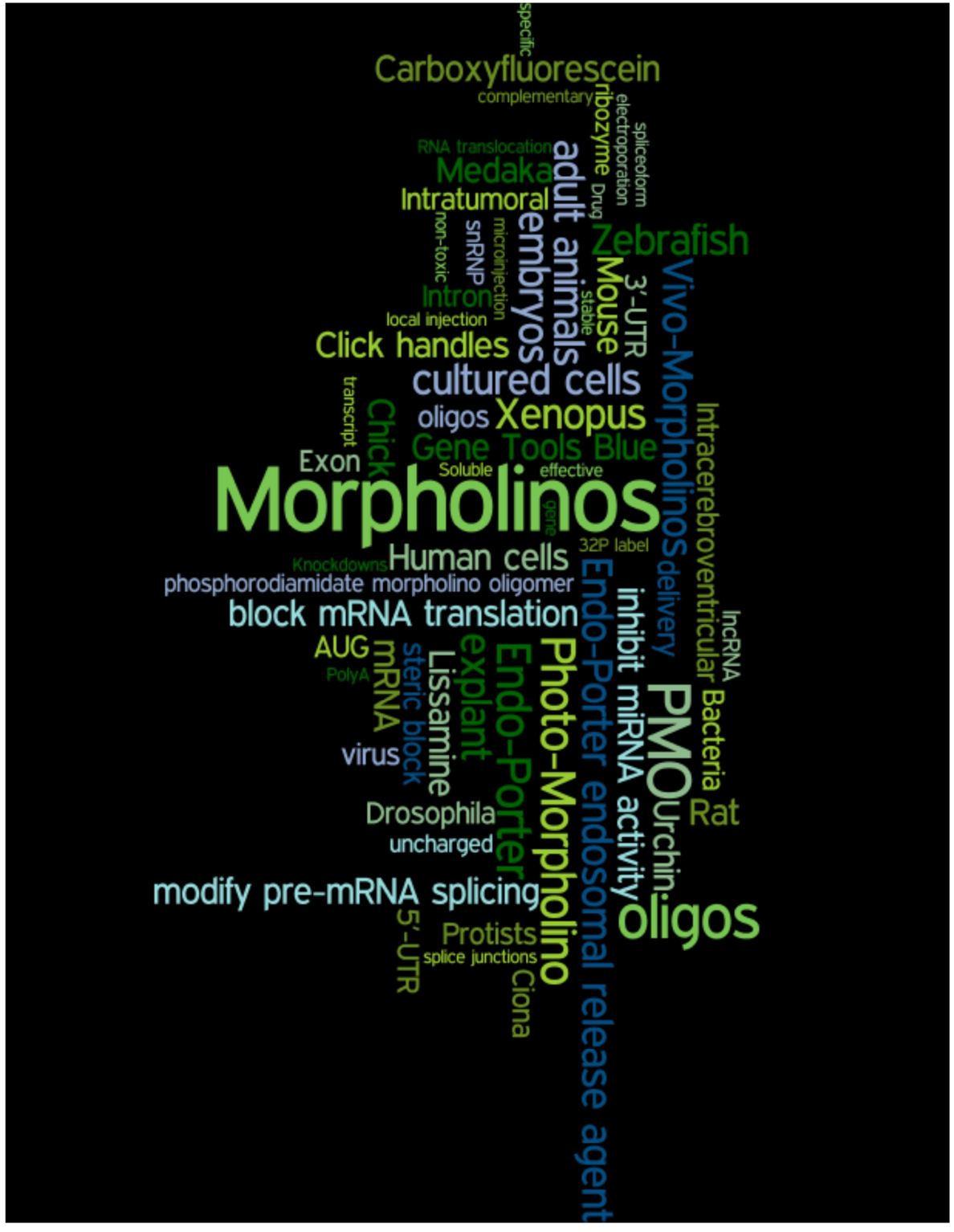
To modify splicing, Morpholinos are either targeted to splice junctions or to the binding sites of splice-regulatory proteins. Here weôll describe targeting splice junctions. A 25-base Morpholino is targeted to the end of an intron as well as 0 to 10 bases of exonic sequence. Targeting the very first or very last splice junction typically results in insertion of the adjacent intron.



Targeting any other splice junction typically results in deletion of the adjacent exon. Other results can occur, such as double-exon-skipping or activation of a cryptic splice site causing partial intron insertions or partial exon deletions. Sometimes two products are produced, for example some transcripts with a clean exon deletion and some with a partial exon deletion.



For help with targeting, see our website (ñOrderingò) or go directly to https://oligodesign.gene-tools.com/



Morpholino oligos
and delivery systems

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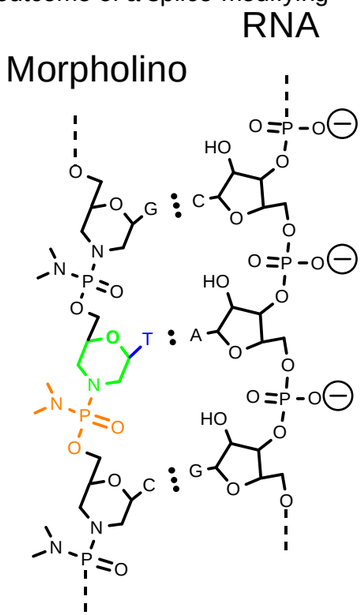
Morpholino oligos are tools for blocking sites on RNA. When a Morpholino binds to a complementary target site on RNA, other macromolecules cannot access the target site. Morpholinos have exquisite specificity for their target sequence, enough to allow targeting RNA in developing embryos. They have high affinity for complementary RNA and can invade RNA secondary structure. Morpholinos do not degrade their targets, instead acting by steric blockade of the target. Their backbone is non-toxic.

Depending on where they are targeting, Morpholinos can block translation, modify splicing, block miRNA maturation and activity, change poly-A tailing, inhibit directed RNA translocation and more.

To block translation, an oligo is targeted to the 5ôUTR through the first part of the coding sequence, with at least part of the oligo target at or upstream of the translation start site. This blocks the movement of the small subunit of the ribosome from the 5êcap to the start codon.

To modify splicing, an oligo can be targeted to the intron at a splice junction or to the binding site of a splice regulatory protein. By preventing a snRNP from binding to the intron near the splice junction, the spliceosome is forced to redirect splicing to the next available snRNP. Modifying splicing allows loss-of-function experiments, characterization of specific exon function or eliminating expression of a particular splice variant or splice mutant. Using reverse-transcriptase PCR, the outcome of a splice-modifying experiment can be assessed without antibodies.

To inhibit microRNA activity, an oligo is targeted to the pri-miRNA hairpin or to the microRNA response element on an mRNA. When a Morpholino invades the pri-miRNA hairpin that structure is no longer a substrate for dsRNA processing nucleases such as Drosha or Dicer. When the microRNA response element is blocked, RISC will no longer bind that element and inhibit translation of the mRNA.



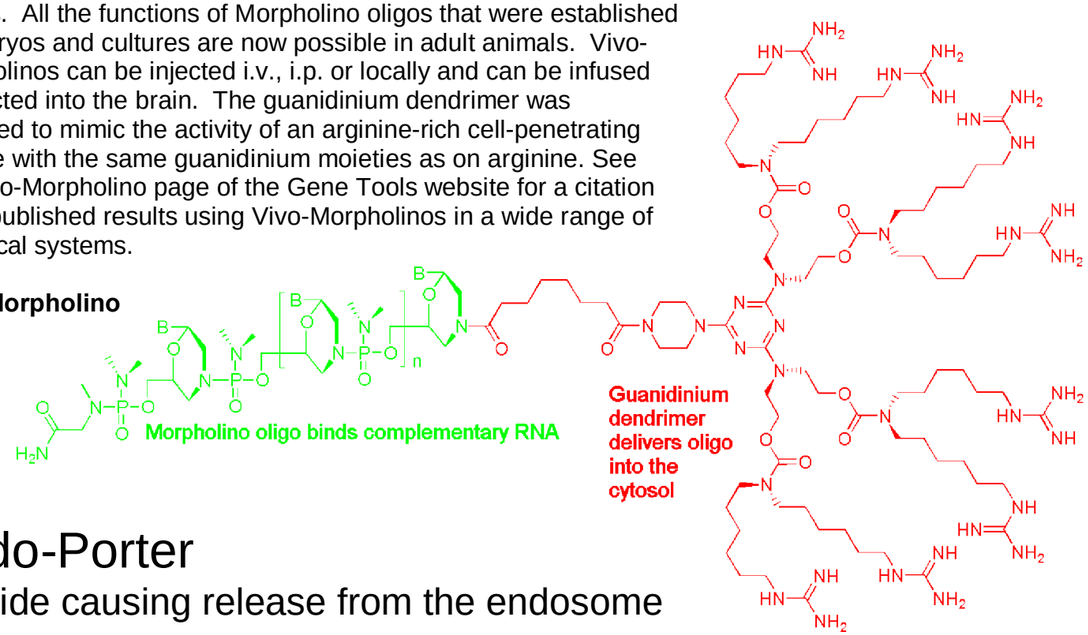
Morpholinos are not nucleic acids. Because the uncharged backbone of a Morpholino oligo is not recognized by any cellular enzymes or signaling proteins, a Morpholino is completely stable to nucleases and does not trigger an innate immune response. Six-membered **Morpholine rings** replace ribose or deoxyribose, non-ionic **phosphorodiamidate intersubunit linkages** replace anionic phosphates, and the standard **nucleobases** (adenine, cytosine, thymine and guanine) are suitably positioned by the backbone to strongly and specifically bind to their RNA target. Morpholino oligos are usually manufactured as 25-mers and are composed entirely of the Morpholino subunits.

Gene Tools provides Ph.D.-level customer support (custsupport@gene-tools.com) and a design service at no cost (oligodesign.gene-tools.com).

Vivo-Morpholinos
One molecule combines antisense and delivery activities

Designed for injection into adult organisms, Vivo-Morpholinos are also effective in cultured cells. Our cytosolic delivery moiety brings covalently-linked Morpholinos into the cytosolic and nuclear compartment of cells. All the functions of Morpholino oligos that were established in embryos and cultures are now possible in adult animals. Vivo-Morpholinos can be injected i.v., i.p. or locally and can be infused or injected into the brain. The guanidinium dendrimer was designed to mimic the activity of an arginine-rich cell-penetrating peptide with the same guanidinium moieties as on arginine. See the Vivo-Morpholino page of the Gene Tools website for a citation list of published results using Vivo-Morpholinos in a wide range of biological systems.

Vivo-Morpholino

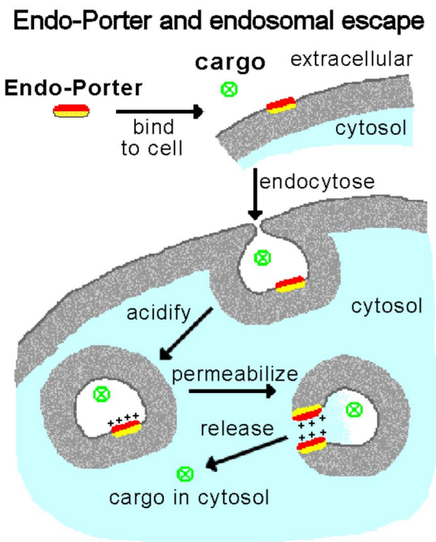


Endo-Porter
Peptide causing release from the endosome

Delivering large molecules into the cytosol of animal cells without damaging the cells has been one of the toughest challenges in biology; Endo-Porter meets that challenge. Endo-Porter is a weak-base amphiphilic peptide that delivers substances (cargo) into the cytosol of cells by an endocytosis-mediated process that avoids damaging the plasma membrane of the cell. This prevents the toxicity typical of most delivery techniques.

Endo-Porter can deliver Morpholinos and other molecules like peptides, proteins and dyes, allowing endocytosed cargo to pass from the endosome into the cytosol of the cell. It is effective with both adherent and non-adherent cells and in 10% serum. It can remain in contact with cells indefinitely and cargo can be delivered repeatedly or continually from any desired extracellular concentration.

Surprisingly simple to use in cultured cells:
Replace spent culture media with fresh, add cargo & mix, pipet in Endo-Porter & immediately swirl to disperse.



Endo-Porter is shipped in DMSO (most effective formulation). Aqueous Endo-Porter is also available.